

What is claimed:

1. An in vivo method of incorporating a polynucleotide into a male vertebrate's germ cells, comprising

administering to a male vertebrate's gonads a transfection mixture comprising at least one polynucleotide encoding a desired trait or product, and at least one transfecting agent, and optionally a genetic selection marker, and under conditions effective to reach the vertebrate's germ cells or precursors thereof; and

allowing the polynucleotide encoding a desired trait or product to be taken up by, and released into, the germ cells or precursors thereof.

2. The method of claim 1, further comprising allowing the incorporation of the released polynucleotide into the genome of the germ cells.

3. The method of claim 1 wherein the transfecting agent is selected from the group consisting of liposomes, viral vectors, transferrin-polylysine enhanced viral vectors, retroviral vectors, lentiviral vectors, and uptake enhancing DNA segments, or comprises a mixture of any members of said group.

4. The method of claim 3, wherein the transfecting agent comprises a viral vector selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, human immunodeficiency virus vectors, lentiviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that facilitate polynucleotide uptake by and release into the cytoplasm of germ cells, or comprises an operative fragment of- or mixture of any members of said group.

5. The method of claim 1, wherein the transfecting agent comprises an

adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

6. The method of claim 1, wherein the transfecting agent comprises a lipid transfecting agent.

7. The method of claim 1, wherein the transfecting agent further comprises a male-germ-cell-targeting molecule.

8. The method of claim 7, wherein the male-germ-cell-targeting molecule is specific for targeting spermatogonia, and is a c-kit ligand.

9. The method of claim 1, where the transfection mixture further comprises an immunosuppressing agent.

10. The method of claim 9, wherein the immunosuppressing agent is selected from the group consisting of cyclosporin and corticosteroids, and the agent is administered systemically.

11. The method of claim 1, wherein the transfection mixture is administered by injection.

12. The method of claim 11, where injection comprises percutaneous injection into the vertebrate's testis.

13. The method of claim 1, wherein the transfection mixture is administered into the vertebrate's testis.

14. The method of claim 13, wherein the transfection mixture is directly administered into the vertebrate's vas efferens.

15. The method of claim 13, wherein the transfection mixture is directly administered into a seminiferous tubule of the vertebrate's testis.

16. The method of claim 1, wherein the transfection mixture is directly administered into the rete of the vertebrate's testis.

17. The method of claim 1, wherein the vertebrate is a mammal.

18. The method of claim 17, wherein the mammal is a human.

19. The method of claim 17, wherein the mammal is selected from the group consisting of human and non-human primates, farm mammals, and marine mammals.

20. The method of claim 19, wherein the farm mammal is selected from the group consisting of swine, equines, ovines and bovines.

21. The method of claim 1, wherein the vertebrate is a bird selected from the group consisting of ducks, geese, turkeys and chickens.

22. The method of claim 1, wherein the vertebrate is selected from the group consisting of wild and domesticated vertebrates.

23. A gene therapy method, comprising the method of claim 1, wherein the polynucleotide encoding a desired trait or product is derived from the same species as the male vertebrate.

24. A non-human transgenic vertebrate produced by the method of claim 1, or progeny thereof, wherein the polynucleotide encoding a desired trait or product is derived from any genome.

25. The non-human transgenic vertebrate of claim 24, comprising native germ cells carrying in their genome at least one xenogeneic polynucleotide.

26. The non-human transgenic vertebrate of claim 25, wherein the polynucleotide comprises at least one biologically functional gene.

27. The non-human transgenic vertebrate of claim 24, being a male.

28. The progeny resulting from breeding the non-human transgenic vertebrate of claim 27, with a female of the same species.

29. A non-human vertebrate, carrying in its germ cells at least one xenogeneic polynucleotide sequence, said non-human vertebrate being obtained by breeding the vertebrate of claim 24, or progeny thereof, with a member of the opposite sex of the same species, and selecting the bred progeny for the presence of the transfected xenogeneic polynucleotide.

30. The non-human vertebrate of claim 29, which is selected from the group consisting of mammals and birds.

31. The non-human vertebrate of claim 30, which is a mammal selected from the group consisting of humans and non-human primates, canines, felines, swine, farm and marine mammals, pachyderms, equines, murine, ovines and bovine, or a bird selected from the group consisting of ducks, geese, turkeys and chickens.

- [illegible]

41. A method of isolating or selecting a male germ cell transfected with at least one polynucleotide encoding a desired trait or product and at least one genetic selection marker, comprising

the method of claim 1, wherein the transfection mixture comprises at least one genetic selection marker; and

isolating or selecting a transfected male germ cell with the aid of the genetic selection marker.

42. A method of transferring maturing male germ cells transfected with at least one polynucleotide encoding a desired trait or product to the testis of a recipient male vertebrate, comprising

isolating or selecting maturing male germ cells carrying at least one polynucleotide encoding a desired trait or product and at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate by the method of claim 41;

administering the germ cells, thus isolated or selected, to a testis of a recipient male vertebrate; and

allowing the administered germ cells to lodge in a seminiferous tubule of the recipient male vertebrate.

43. A method of transferring autologous germ and support cells to the testis of a vertebrate, comprising the method of claim 42, wherein the donor vertebrate is the same as the recipient vertebrate.

44. The method of claim 41, further comprising the step of incorporating into the genome of the germ cell the polynucleotide encoding a desired trait or product.

45. The method of claim 41, wherein the transfected male germ cell comprises an undifferentiated male germ cell.

46. The method of claim 41, wherein transfection is conducted under conditions of temperature of about 25°C to about 38°C.

47. The method of claim 41, wherein the transfecting agent is selected from the group consisting of liposomes, viral vectors, transferrin-polylysine enhanced viral vectors, retroviral vectors, lentiviral vectors, and other uptake enhancing DNA segments, or comprises a mixture of any members of said group.

48. The method of claim 47, wherein the transfecting agent comprises a viral vector selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, human immunodeficiency virus vectors, lentiviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that facilitate polynucleotide uptake by and release into the cytoplasm of germ cells, or said transfecting agent comprises an operative fragment of- or mixture of any members of said group.

49. The method of claim 47, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

50. The method of claim 41, wherein the polynucleotide encoding a desired trait or product is in the form of a complex with a viral vector.

51. The method of claim 41, wherein the transfecting agent comprises a lipid transfecting agent.

52. The method of claim 42, wherein the transfecting agent further comprises an agent selected from the group consisting of a male-germ-cell-targeting molecule and at least one genetic selection marker.

53. The method of claim 52, wherein the male-germ-cell-targeting molecule is specifically targeted to spermatogonia and comprises a c-kit ligand; and

the genetic selection marker comprises a gene encoding a detectable product, expression of said gene being driven by a spermatogonia-specific promoter, said promoter being selected from the group consisting of c-kit promoter, b-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, and FRMI (from fragile X site) promoter.

54. The method of claim 41, wherein the vertebrate is a mammal.

55. The method of claim 54, wherein the mammal is a human.

56. The method of claim 54, wherein the mammal is selected from the group consisting of human and non-human primates and farm and marine mammals.

57. The method of claim 42, wherein the polynucleotide encoding a desired trait or product is derived from the same species of vertebrate as the recipient vertebrate.

58. The method of claim 42, wherein the vertebrate is selected from the group consisting of wild and domesticated vertebrates.

59. The method of claim 41, wherein the polynucleotide encoding a desired trait or product is derived from a mammal selected from the group consisting of human and non-human primates, canines, felines, swines, farm mammals, pachyderms, marine mammals, equines, murine, ovine and bovine, or from a bird selected from the group consisting of ducks, geese, turkeys and chickens.

FOOTNOTES

60. The method of claim 59, wherein the polynucleotide is derived from a human.

61. A non-human transgenic vertebrate, comprising native germ cells carrying in their genomes at least one xenogeneic polynucleotide, said transgenic vertebrate being the recipient male vertebrate of the method of claim 42, or progeny thereof.

62. The non-human transgenic vertebrate of claim 61, wherein the polynucleotide comprises at least one biologically functional gene.

63. The non-human transgenic vertebrate of claim 62, being a male.

64. The non-human transgenic vertebrate of claim 63, harboring native male germ cells transfected with a xenogeneic polynucleotide.

65. The progeny resulting from breeding the non-human transgenic vertebrate of claim 63 or progeny thereof, with a female of the same species.

66. A non-human vertebrate, carrying in its germ cells at least one xenogeneic polynucleotide sequence, obtained by breeding the vertebrate of claim 61 or progeny thereof, with a member of the opposite sex of the same species, and selecting the bred progeny for the presence of the transfected xenogeneic polynucleotide.

67. The non-human vertebrate of claim 66, which is selected from the group consisting of mammals and birds.

68. The non-human vertebrate of claim 67, which is a mammal selected from the group consisting of humans and non-human primates, canines, felines, swine, farm and marine mammals, pachyderms, equines, murine, ovines and bovine, and a bird selected from

4005466-1-130

the group consisting of ducks, geese, turkeys and chickens.

69. The non-human vertebrate of claim 67, which is a bird selected from the group consisting of ducks, geese, turkeys and chickens.

70. The non-human vertebrate of claim 67, wherein the mammal is a farm or marine mammal.

71. The non-human vertebrate of claim 68, wherein the mammal is a bull.

72. The non-human vertebrate of claim 68, wherein the mammal is a pig.

73. The non-human vertebrate of claim 66, which is selected from the group consisting of wild and domesticated animals.

74. A germ cell obtained from a vertebrate of claims 24 or 61 comprising a native germ cell carrying in its genome at least one xenogeneic polynucleotide.

75. Vertebrate semen comprising the germ cell of claim 74.

76. A gene therapy method, comprising the method of claim 42, wherein the polynucleotide encoding a desired trait or product is derived from the same species of vertebrate as the recipient vertebrate.

77. A non-human transgenic vertebrate produced by the method of claim 42, wherein the polynucleotide encoding a desired trait or product is derived from any genome.

78. An in vitro method of incorporating at least one polynucleotide encoding a

COPIES 1054500

desired trait into a maturing male germ cell, comprising

obtaining a maturing male germ cell from a vertebrate;

5 transfecting the germ cell in vitro with at least one polynucleotide encoding a desired trait in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection marker, at about or below the vertebrate's body temperature and for a transfection-effective period of time; and

allowing the polynucleotide encoding a desired trait to be taken up by, and released into the germ cell.

79. The method of claim 78, further comprising allowing the incorporation of the released polynucleotide into the genome of the germ cell.

80. The method of claim 78, wherein the encoding a desired trait is incorporated into the vertebrate germ cell's genome.

81. The method of claim 78, wherein the maturing male germ cell comprises a spermatogonia or other undifferentiated male germ cell.

82. The method of claim 78, wherein the transfection is conducted under conditions of temperature of about 25°C to about 38°C.

83. The method of claim 78, wherein the transfecting agent is selected from the group consisting of liposomes, viral vectors, transferrin-polylysine enhanced viral vectors, retroviral vectors, lentiviral vectors, and other uptake enhancing DNA segments, or comprises a mixture of any members of said group.

84. The method of claim 83, wherein the transfecting agent comprises a viral vector selected from the group consisting of retroviral vectors, adenoviral vectors,

4003436-1104

transferrin-polylysine enhanced adenoviral vectors, human immunodeficiency virus vectors, lentiviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that enhance polynucleotide uptake by and release into the cytoplasm of germ cells, or said transfecting agent comprises an operative fragment of- or mixture of any members of said group.

85. The method of claim 84, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide encoding a desired trait is operatively linked to the vector.

86. The method of claim 78, wherein the polynucleotide encoding a desired trait is in the form of a complex with a viral vector.

87. The method of claim 78, wherein the transfecting agent comprises a lipid transfecting agent.

88. The method of claim 78, wherein
the transfecting agent further comprises an agent selected from the group consisting of a male-germ-cell-targeting molecule and at least one genetic selection marker; and
the method further comprises isolating or selecting a maturing male germ cell carrying at least one polynucleotide encoding a desired trait or product and at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate with the aid of the genetic selection marker.

89. The method of claim 88, wherein
the male-germ-cell-targeting molecule is specifically targeted to spermatogonia and comprises a c-kit ligand, and
the genetic selection marker comprises a gene expressing a detectable product, driven by a spermatogonia-specific promoter selected from the group consisting of c-kit promoter,

b-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, and FRMI (from fragile X site) promoter.

90. The method of claim 78, wherein the vertebrate is a mammal.

91. The method of claim 90, wherein the mammal is a human.

92. The method of claim 90, wherein the mammal is selected from the group consisting of human and non-human primates and farm and marine mammals.

93. The method of claim 78, wherein the polynucleotide encoding a desired trait is derived from the same vertebrate species as the maturing germ cell.

94. The method of claim 78, wherein the vertebrate is selected from the group consisting of wild and domesticated vertebrates.

95. The method of claim 78, wherein the polynucleotide encoding a desired trait is derived from a mammal selected from the group consisting of human and non-human primates, canines, felines, swines, farm mammals, pachyderms, marine mammals, equines, murine, ovine and bovine, or from a bird selected from the group consisting of ducks, geese, turkeys and chickens.

96. The method of claim 95, wherein the polynucleotide is derived from a human.

97. A non-human transgenic vertebrate, or its progeny, comprising a native germ cell carrying in its genome at least one xenogeneic polynucleotide, said polynucleotide having been incorporated into the genome of said germ cell through the method of claim 78.

98. The non-human transgenic vertebrate of claim 97, wherein the polynucleotide comprises at least one biologically functional gene.

99. The non-human transgenic vertebrate of claim 98, being a male.

100. The non-human transgenic vertebrate of claim 99, harboring native male germ cells transfected with a xenogeneic polynucleotide.

101. The progeny resulting from breeding the non-human transgenic vertebrate of claim 99 or progeny thereof, with a female of the same species.

102. A non-human vertebrate, carrying in its germ cells at least one xenogeneic polynucleotide sequence, said vertebrate obtained by breeding the vertebrate of claim 98 or progeny thereof, with a member of the opposite sex of the same species, and selecting the bred progeny for the presence of the transfected xenogeneic polynucleotide.

103. The non-human vertebrate of claim 102, which is selected from the group consisting of mammals and birds.

104. The non-human vertebrate of claim 103, which is a mammal selected from the group consisting of humans and non-human primates, canines, felines, swine, farm and marine mammals, pachyderms, equines, murine, ovines and bovine, or a bird selected from the group consisting of ducks, geese, turkeys and chickens.

105. The non-human vertebrate of claim 104, which is a bird selected from the group consisting of ducks, geese, turkeys and chickens.

106. The non-human vertebrate of claim 104, wherein the mammal is a farm or

marine mammal.

107. The non-human vertebrate of claim 104, wherein the mammal is a bull.

108. The non-human vertebrate of claim 104, wherein the mammal is a pig.

109. The non-human vertebrate of claim 102, which is selected from the group consisting of wild and domesticated animals.

110. A germ cell obtained from the vertebrate of claim 97, or its progeny.

111. Vertebrate semen comprising a plurality of the germ cells obtained from the vertebrate of claim 98.

112. A gene therapy method, comprising the method of claim 78; further comprising the step of introducing said transfected male germ cell into the testis of a recipient vertebrate, wherein the polynucleotide encoding a desired trait is derived from the same vertebrate species as the recipient vertebrate.

113. A non-human transgenic vertebrate produced by the method of claim 78, wherein the polynucleotide encoding a desired trait is derived from any genome.

114. A kit for the transfection and storage of a male vertebrate's germ cells, comprising a transfection mixture, said transfection mixture comprising at least one transfecting agent, and optionally a genetic selection marker, whereby said kit may be used to transfect and store said germ cells in a viable condition.

115. The kit of Claim 114, wherein the transfecting agent is selected from the

group consisting of liposomes, viral vectors, transferrin-polylysine enhanced viral vectors, retroviral vectors, lentiviral vectors, and uptake enhancing DNA segments, or comprises a mixture of any members of said group.

116. The kit of Claim 114, wherein the transfecting agent comprises a viral vector selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, human immunodeficiency virus vectors, lentiviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, DNAs that facilitate polynucleotide uptake by and release into the cytoplasm of germ cells, or comprises an operative fragment of- or mixture of any members of said group.

117. The kit of Claim 114, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

118. The kit of Claim 114, wherein the transfecting agent comprises a lipid transfecting agent.

119. The kit of Claim 114, wherein the transfecting agent further comprises a male-germ-cell-targeting molecule.

120. The kit of Claim 119, wherein the male-germ-cell-targeting molecule is specific for targeting spermatogonia and comprises a c-kit ligand.

121. The kit of Claim 114, where the transfection mixture further comprises an immunosuppressing agent.

122. The kit of Claim 121, wherein the immunosuppressing agent is selected from

the group consisting of cyclosporin and corticosteroids.

123. The kit of Claim 119, wherein the male-germ-cell-targeting molecule is specifically targeted to spermatogonia and comprises a c-kit ligand; and
the genetic selection marker comprises a gene expressing a detectable product driven by a spermatogonia-specific promoter.

124. The kit of Claim 119, wherein the male-germ-cell-targeting molecule is specifically targeted to spermatogonia and comprises a c-kit ligand; and
the genetic selection marker comprises a gene expressing a detectable product, driven by a spermatogonia-specific promoter, said promoter selected from the group consisting of c-kit promoter, b-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, and FRMI (from fragile X site) promoter.

125. The kit of Claim 114, wherein at least one polynucleotide comprises at least one polynucleotide sequence encoding a genetic selection marker.

126. The kit of Claim 114, further comprising a cryoprotectant.

127. The germ cell as in any of Claims 35, 36, 37, 74, or 110, wherein said germ cell has been cryopreserved in a viable and functional condition.

128. A transgenic male germ cell produced by the method of any of Claims 17, 18, 19, 20, 21, or 22, wherein the transgenic male germ cell has been cryopreserved in a viable and functional condition.

129. A transgenic male germ cell produced by the method of any of Claims 54,

55, 56, or 58, wherein the transgenic male germ cell has been cryopreserved in a viable and functional condition.

130. A transgenic male germ cell produced by the method of any of Claims 90, 91, 92, or 94, wherein the transgenic male germ cell has been cryopreserved in a viable and functional condition.

131. The method of any of Claims 1 or 78, wherein the polynucleotide encoding a desired trait or product is operatively linked to a germ cell-specific promoter.

132. The method of any of Claims 41, 78, or 88, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

133. The method of Claim 42, wherein support cells are co-administered to a testis along with isolated or selected germ cells.

134. The method of Claim 42, wherein transfected support cells are isolated or selected, and co-administered to a testis of a recipient male vertebrate along with said isolated or selected germ cells.